

Germline variants in DNA repair genes, including *BRCA1/2*, may cause familial myeloproliferative neoplasms

Miriam Elbracht,¹ Robert Meyer,¹ Kim Kricheldorf,² Deniz Gezer,² Thomas Eggermann,¹ Beate Betz,³ Ingo Kurth,¹ Lino L. Teichmann,⁴ Tim H. Brümmendorf,² Ulrich Germing,⁵ Susanne Isfort,² and Steffen Koschmieder²

¹Institute of Human Genetics, Medical Faculty, RWTH Aachen University, Aachen, Germany; ²Department of Hematology, Oncology, Hemostaseology and Stem Cell Transplantation, Medical Faculty, RWTH Aachen University, Aachen, Germany; ³Institute for Human Genetics, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; ⁴Department of Hematology and Oncology, University Hospital Bonn, Bonn, Germany; and ⁵Department of Hematology, Oncology and Clinical Immunology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Key Points

- Germline variants in DNA repair pathway genes (eg, *BRCA1/2*) can cause familial MPNs and should be included in diagnostic workup.
- Germline predisposition in MPNs might involve higher leukemic transformation and require novel therapeutic options (eg, PARP inhibitors).

The molecular causes of myeloproliferative neoplasms (MPNs) have not yet been fully elucidated. Approximately 7% to 8% of the patients carry predisposing genetic germline variants that lead to driver mutations, which enhance JAK-STAT signaling. To identify additional predisposing genetic germline variants, we performed whole-exome sequencing in 5 families, each with parent-child or sibling pairs affected by MPNs and carrying the somatic *JAK2* V617F mutation. In 4 families, we detected rare germline variants in known tumor predisposition genes of the DNA repair pathway, including the highly penetrant *BRCA1* and *BRCA2* genes. The identification of an underlying hereditary tumor predisposition is of major relevance for the individual patients as well as for their families in the context of therapeutic options and preventive care. Two patients with essential thrombocythemia or polycythemia vera experienced progression to acute myeloid leukemia, which may suggest a high risk of leukemic transformation in these familial MPNs. Our study demonstrates the relevance of genetic germline diagnostics in elucidating the causes of MPNs and suggests novel therapeutic options (eg, PARP inhibitors) in MPNs. Furthermore, we uncover a broader tumor spectrum upon the detection of a germline mutation in genes of the DNA repair pathway.

Introduction

Myeloproliferative neoplasms (MPNs) typically occur sporadically and are caused by somatic driver mutations in the *JAK2* (Janus kinase 2), *CALR* (calreticulin), and *MPL* (thrombopoietin receptor) genes. However, familial clustering occurs in 7% to 8% of cases, with relatives of MPN patients exhibiting a five- to sevenfold MPN risk.¹⁻³ Interestingly, driver mutations and MPN phenotypes vary between family members,³ suggesting a common germline predisposition for the subsequent driver mutations. Known predisposing germline genetic variations and associated MPN risks include: *JAK2* 46/1 haplotype (up to fourfold)⁴⁻⁶; telomerase reverse transcriptase *TERT* promoter variant (rs2736100) (2-fold),⁷ duplication of *ATG2B* (autophagy related 2B) and *GSKIP* (GSK3B interacting protein; high risk of progression from essential thrombocythemia [ET] to myelofibrosis or leukemia),⁸ and germline *RBBP6* (retinoblastoma binding protein 6) mutations (~5% of familial MPN cases).⁹

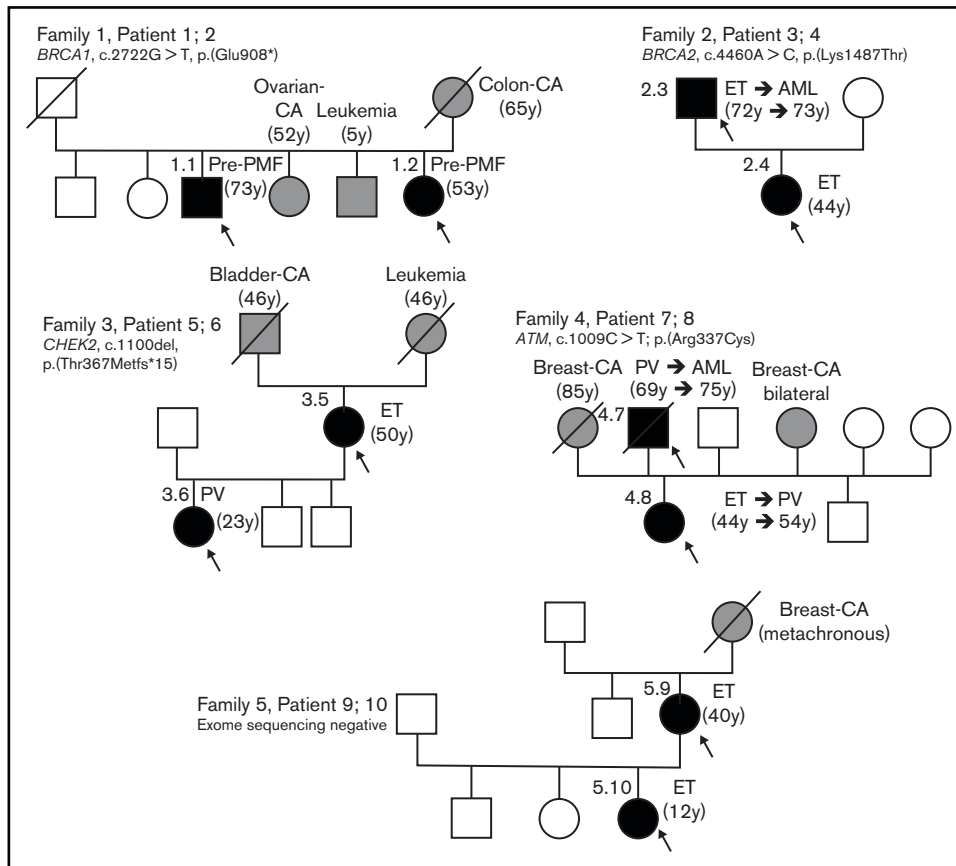


Figure 1. Pedigrees of the 5 families with familial *JAK2* V617F⁺ MPNs. Arrows indicate the affected patients. A line through the symbol indicates a patient who died. Family members affected by cancer (CA) are marked in gray. MPN subtype (essential thrombocythemia [ET], polycythemia vera [PV], prefibrotic primary myelofibrosis [pre-PMF]), age at MPN onset and progression, and progression to PV or acute myeloid leukemia (AML) are indicated for each patient. The germline gene variant for each family is depicted above each pedigree. In patient 4, the diagnosis was ET, but pre-PMF could not be excluded.

Methods

Our study was approved by the ethics committee of the Medical Faculty of RWTH Aachen University (EK306) and was conducted in accordance with the Declaration of Helsinki. To identify additional predisposing germline factors in familial MPNs, we performed whole-exome sequencing (WES) in 5 families affected by MPNs and carrying a somatic *JAK2* V617F (c.1849G>T) mutation (Figure 1). WES was carried out by analyzing the DNA of peripheral blood samples on a NextSeq500 Sequencer with 2 × 75 cycles on a high-output flow cell (Illumina, San Diego, CA). A probe-based capture method was used to enrich the target regions (Integrated DNA Technologies, Coraville, IA, or Nextera Rapid Capture Exome [version 1.2]; Illumina). Alignment to the reference genome (hg19 or hg38) and variant calling were performed using an in-house pipeline based on SeqMule (<http://seqmule.openbioinformatics.org/en/latest>). Variant analysis and prioritization were performed using Kggseq software (<http://pmglab.top/kggseq/>). Variants of interest were confirmed by Sanger sequencing on an ABI3500 platform (Applied Biosystems, Foster City, CA); to exclude a somatic origin, DNA from hair roots and/or finger nails was used for Sanger sequencing.

Results and discussion

In 4 of 5 families, we found heterozygous germline variants in DNA repair genes associated with hereditary cancer predisposition

syndromes, especially hereditary breast and ovarian cancers (Figure 1).

In family 1, a brother (1.1) and sister (1.2) with prefibrotic myelofibrosis carried a heterozygous loss-of-function mutation in the *BRCA1* (breast cancer 1) gene (OMIM*113705, transcript NM_007294.3). The sister (1.2) was first diagnosed with MPN at age 53 years; she later developed uterine cancer at age 61 years. The variant c.2722G>T, p.(Glu908*) leads to a premature translational stop and is not listed in the Genome Aggregation Database (gnomAD). It is reported once (1 in 8600 alleles) in the Exome Sequencing Project database (as of 1 February 2019) and has been described in patients with hereditary breast or ovarian cancer.¹⁰ According to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines, the variant must be classified as pathogenic. Family history of the patients in family 1 was conspicuous for cancer, with deaths resulting from colorectal cancer in the mother at age 65 years and 1 brother at age 77 years, ovarian cancer in 1 sister at age 52 years, and leukemia in 1 brother at age 5 years.

In family 2, a father (2.3) and daughter (2.4) presented with ET. Subsequently, the father experienced progression to AML. In both individuals, a missense variant in the *BRCA2* (breast cancer 2) gene (OMIM*600185, transcript NM_000059.3) was identified. The variant c.4460A>C, p.(Lys1487Thr) has not yet been described in large

genome databases (eg, gnomAD as of 23 December 2019) or in the literature. High conservation of the affected amino acid argues for its functional relevance. Bioinformatic prediction programs predicted the effect of the variant inconsistently: SIFT (version 6.2.0), tolerated (score, 0.57; median, 2.68); MutationTaster (version 2013), disease causing (probability, 0.971); and PolyPhen-2 (HumVar), possibly damaging (score, 0.796). Formally, the variant must be classified as a variant of uncertain significance according to the ACMG criteria. Family history of the rather small family was unremarkable. Interestingly, patient 2.3 showed an excellent response to decitabine monotherapy for AML.

In a mother (3.5) and daughter (3.6) of family 3 who had ET (although prefibrotic myelofibrosis could not be completely ruled out) and PV, respectively, the pathogenic frameshift variant c.1100del (p. Thr367Metfs*15) in the *CHEK2* (checkpoint kinase 2) gene (OMIM*604373, transcript NM_007194.4) was identified, likely leading to loss of protein function. It is listed in gnomAD (as of 3 October 2020) with a frequency of 0.21% and has been described in association with tumor predisposition as the most common pathogenic *CHEK2* mutation.¹¹ According to the ACMG guidelines, the variant is classified as pathogenic. A predisposition to MPNs caused by this variant has already been postulated in the literature.¹² The patient's (3.5) mother (grandmother of patient 3.6) died as a result of leukemia not further specified at age 46 years. The father of patient 3.5 (grandfather of patient 3.6) died as a result of bladder cancer at age 75 years. Her cousin died as a result of ovarian cancer in young adulthood.

In family 4, the father (4.7), affected by prostate carcinoma at age 64 years (treated by surgery only), was diagnosed with PV at age 69 years, which subsequently progressed to AML at age 75 years. His daughter (4.8) suffered from ET, which subsequently progressed to PV. Both (4.7 and 4.8) carried a heterozygous missense variant in the tumor predisposition gene *ATM* (ataxia-teleangiectasia mutated; OMIM*607585, transcript NM_000051.3). The variant c.1009C>T, p.(Arg337Cys) is listed with a low frequency of 0.0092% (15 December 2020). Bioinformatically, the alteration is classified as likely disease causing (PolyPhen-2, MutationTaster, and SIFT). Formally, the variant must be classified as a variant of uncertain significance according to the ACMG criteria. Interestingly, family history was conspicuous for breast cancer. On the father's side, 1 sister developed bilateral breast cancer, and another sister died at age 85 years as a result of breast cancer.

In a mother (5.9) and daughter (5.10) age 11 years of family 5, both affected by ET, we did not identify any pathogenic or likely pathogenic variant in the applied WES approach or *BRCA1/2* multiplex ligation-dependent probe amplification. Submicroscopic chromosomal imbalances were excluded in the patients of family 5 by single-nucleotide polymorphism array analysis (CytoScan HD array; Affymetrix, Santa Clara, CA). Family history was not unremarkable. The deceased mother of 5.9 (grandmother of 5.10) had been diagnosed with metachronous breast cancer. Further information regarding this was not available.

While an association of germline variants in *ATM*⁺, *CHEK2*⁺, and *JAK2* V617F⁺ MPNs has already been postulated,¹² such an association, to our knowledge, has not been demonstrated for the high-risk hereditary cancer genes *BRCA1* and *BRCA2*. Nevertheless, germline heterozygous pathogenic variants in genes of the Fanconi anemia–*BRCA* pathway are known to predispose for hematological malignancies, which may include an increased risk for MPNs.¹³ In

addition, we have published data demonstrating that JAK inhibition by ruxolitinib decreases the *BRCA* protein in *JAK2* V617F–mutant MPN cells and renders them more susceptible to PARP inhibitor–induced apoptosis,¹⁴ supporting a functional interaction between the Fanconi anemia–*BRCA* and JAK–STAT pathways. These results suggest that *BRCA* haploinsufficiency may predispose patients to an MPN but also render them more susceptible to combined JAK and PARP inhibition. The exact mechanisms are not yet understood but could involve germline predisposition to genomic instability and subsequent acquisition of oncogenic mutations, such as *JAK2* V617F, which in turn stimulate increased reactive oxygen species production and again result in genomic instability. Interestingly, mutant *CHEK2* has already been demonstrated to enhance stem cell expansion in both the absence and presence of genotoxic stress.¹⁵

The identification of an underlying hereditary tumor predisposition has therapeutic consequences for patients as well as their families. The detection of a pathogenic germline variant in the high-penetrance genes *BRCA1* and *BRCA2* should prompt the discussion of prophylactic surgery (mastectomy or oophorectomy). The fact that 2 patients with ET or PV experienced progression to AML may suggest a high risk of leukemic transformation and the necessity for closer monitoring of clonal evolution. Germline mutations in DNA repair genes may affect the type of cytoreductive therapy (eg, the cytostatic agent hydroxyurea may be more toxic but also more efficacious than other treatments). Moreover, in the case of a planned allogeneic stem cell transplantation, it may be important to exclude the pathogenic germline mutation in suitable family donors and also to adapt conditioning protocols in the respective patient.

In summary, the data suggest that MPNs are more frequently triggered by germline mutations than previously assumed, and conversely, the spectrum of known tumor predisposition syndromes seems to be broader than anticipated.

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Authorship

Contribution: The study was designed by M.E., L.L.T., and S.K. Data were collected by M.E., R.M., K.K., D.G., U.G., S.I., and S.K. Data were analyzed by all authors. The paper was written by M.E. and S.K. and edited by all authors. All authors approved the final manuscript.

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ORCID profiles: M.E., 0000-0001-5088-1369; R.M., 0000-0002-5693-2216; K.K., 0000-0003-3102-2221; T.E., 0000-0002-8419-0264; I.K., 0000-0002-5642-8378; T.B., 0000-0002-9677-3723; S.K., 0000-0002-1011-8171.

References

1. Rumi E, Passamonti F, Della Porta MG, et al. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. *J Clin Oncol*. 2007;25(35):5630-5635.
2. Landgren O, Goldin LR, Kristinsson SY, Helgadóttir EA, Samuelsson J, Björkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. *Blood*. 2008;112(6):2199-2204.
3. Rumi E, Cazzola M. Advances in understanding the pathogenesis of familial myeloproliferative neoplasms. *Br J Haematol*. 2017;178(5):689-698.
4. Jones AV, Chase A, Silver RT, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):446-449.
5. Kilpivaara O, Mukherjee S, Schram AM, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):455-459.
6. Olcaydu D, Harutyunyan A, Jäger R, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet*. 2009;41(4):450-454.
7. Jäger R, Harutyunyan AS, Rumi E, et al. Common germline variation at the TERT locus contributes to familial clustering of myeloproliferative neoplasms. *Am J Hematol*. 2014;89(12):1107-1110.
8. Saliba J, Saint-Martin C, Di Stefano A, et al. Germline duplication of ATG2B and GSKIP predisposes to familial myeloid malignancies. *Nat Genet*. 2015;47(10):1131-1140.
9. Harutyunyan AS, Giamb Bruno R, Krendl C, et al. Germline RBBP6 mutations in familial myeloproliferative neoplasms. *Blood*. 2016;127(3):362-365.
10. Castéra L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. *Eur J Hum Genet*. 2014;22(11):1305-1313.
11. Apostolou P, Papanotiropoulou I. Current perspectives on CHEK2 mutations in breast cancer. *Breast Cancer (Dove Med Press)*. 2017;9:331-335.
12. Hinds DA, Barnholt KE, Mesa RA, et al. Germ line variants predispose to both JAK2 V617F clonal hematopoiesis and myeloproliferative neoplasms. *Blood*. 2016;128(8):1121-1128.
13. Pouliot GP, Degar J, Hinze L, et al. Fanconi-BRCA pathway mutations in childhood T-cell acute lymphoblastic leukemia. *PLoS One*. 2019;14(11):e0221288.
14. Nieborowska-Skorska M, Maifrede S, Dasgupta Y, et al. Ruxolitinib-induced defects in DNA repair cause sensitivity to PARP inhibitors in myeloproliferative neoplasms. *Blood*. 2017;130(26):2848-2859.
15. Bao EL, Nandakumar SK, Liao X, et al; 23andMe Research Team. Inherited myeloproliferative neoplasm risk affects haematopoietic stem cells. *Nature*. 2020;586(7831):769-775.